High pressure processing (HPP) of pea starch: Effect on the gelatinization properties

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Abstract

High pressure processing (HPP), an emerging technology, can be used to promote gelatinization of starch granules. This phenomenon is highly dependent on the source of starch, pressure level, time and temperature applied as well as the dispersion medium. This work evaluated the effect of HPP (up to 600 MPa/15 min/25°C) on particle size distribution, optical microscopy, differential scanning calorimetry and pasting properties of pea starch. Results showed no difference between control samples and processed ones up to 400 MPa (water dispersion) or all samples dispersed in ethanol, except for the thermal properties at 400 MPa that showed 31% of gelatinization in water dispersion. Samples processed at pressures higher than 500 MPa showed changes on particle size and distribution (increase at 500 MPa and a slight reduction at 600 MPa), and no detected gelatinization enthalpy at DSC. The optical microscopy observation indicated that HPP (≥400 MPa) caused the loss of birefringence. Regarding the pasting properties, the initial viscosity increased from 8 cP at 0 MPa to 34 cP at 600 MPa. All results indicated that HPP can be used to promote “cold gelatinization” on pea starch water dispersion, achieving a specific technological profile and possibly leading to new ingredients.

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1. Introduction

Starch is the main carbohydrate reserve in higher plants and the most important source of energy for humans. Industrially, it has been widely used for numerous applications in various industries, including food and non-food, due to its functional properties, such as dispersion of ingredients, texturizing agent, fat replacer, and mouth feel enhancer (Adebowale, Afolabi, & Olu-Owolabi, 2005; Perez-Pacheco et al., 2014; Wang & Copeland, 2013).

Starch structure and properties of phase transitions are important once they influence viscosity, appearance, texture, waterholding capacity and enzyme digestibility of processed starch based food products (Wang & Copeland, 2012). The physicochemical properties of the starch and thus its applications depend on factors such as the amylose/amylopectin ratio, granule size and shape, degree of polymerisation, diffraction pattern and the differences in crystalline/amorphaous regions of granules, as well as its botanical origin and source (Błaszczyk et al., 2003; Sankhon et al., 2014).

Pea starch is mainly available as a by-product from pea protein extraction, being a relative cheaper source of starch compared to wheat and potato. Pea starch is characterized by its high amylose content (35–65%), fast retrogradation, resistance to shear thinning and high resistant starch content (Wang & Copeland, 2015). Due to its high amylose starch percentage, pea starch is mainly industrially used to obtain flexible films with good mechanical properties and gas barrier (Ratnayake, Hoover, & Warkentin, 2002).

Chemical and physical modifications of starch are commonly employed to obtain starches with special functional properties, increasing its range of use. Although chemically modified starches are available for industrial uses, most industries (especially food and pharmaceutical industries) prefer starches that have been physically altered (heat, moisture, shear, radiation, high pressure processing) due to their relative safety (Adebowale et al., 2005).

High pressure processing (HPP) is a non-thermal emerging technology that subjects a product to high pressures (up to 1000 MPa) for a controlled time and temperature. HPP affects only non-covalent bonds and can cause serious structural damage to biopolymers,
including protein denaturation and starch gelatinization (Balny, Masson, & Heremans, 2002; Hu et al., 2011). In this context, HPP has been employed to gelatinize or physically modify different types of starch dispersions (Li et al., 2011, 2012; Yang, Gu, & Hemar, 2013). Starch granules could be gelatinized completely at room temperature by HPP and the impact of the process in starch gelatinization depends markedly on starch type, treatment pressure, temperature, time and water content (Bauer & Knorr, 2005; Li et al., 2011, 2012). All data relative to HPP on starch were obtained in aqueous media; being not previously established if the HPP can induce changes on starch dispersed in other dispersion media, or if water (hydration) has a central role in the modifications observed in starches after HPP.

Several studies have been carried out with starch processed by HPP (Li et al., 2011, 2012; Yang et al., 2013), however, nothing has been done to verify its effects on Particle Size Distribution (PSD), optical microscopy, Differential Scanning Calorimetry (DSC) and pasting properties on the same study, which disrupts the well understanding of the phenomenon on the starch. Additionally, there are studies on the structure and functionality of starches from peas (Bogracheva, Morris, Ring, & Hedley, 1998; Chung & Liu, 2012; Ratnayake, Hoover, Shahidi, Perera, & Jane, 2001; Wang, Sharp, & Copeland, 2011), but little information is available for starches from peas processed by HPP. In respect to that, Le Bail et al. (2013) observed that HPP (500 MPa; 20 min; 20 or 40 °C) induced gelatinization of several starches including pea starch, lacking a deep evaluation about the process impact on the pea starch molecular structure. Considering the lack of information about HPP effects on pea starch and on starches dispersed in non-aqueous media, this work aimed to evaluate the impact of HPP on pea starch dispersed in water and ethanol by using structural and functional evaluation of the processed sample.

2. Materials and methods

2.1. Pea starch and dispersions preparation

Pea starch (33% of amylose) was obtained from Emsland-Stärke Group - Food Division, Germany. Starch samples were prepared using 4% (w/w) of starch dispersed in distilled water or in ethanol (99.9%), stirred at 25 °C (using magnetic mixer) within 24 h prior to processing.

2.2. High pressure processing (HPP)

A high pressure equipment (QFP 2L - 700 Avure Technologies, OH, USA) with a chamber of 2 L volume, maximum pressure work of 690 MPa and temperature controlled from 10 to 90 °C was used in the assays. Samples were packaged in sealed LDPE-Nylon-LDPE bags (16 μm thickness - TecMaq, Brazil). Processes were performed at 300, 400, 500 and 600 MPa for 15 min at 25 °C. The pressurization rate was about 6 ± 0.5 MPa/s; the depressurization time was almost instantaneous. After process, all samples were distributed in glass petri dishes, cooled down quickly, lyophilized (water dispersion) or air dried (ethanol dispersion) and stored before analysis. All processes were carried out in triplicate.

2.3. Visual observation and optical microscopy observation

Visual observation was conducted in graduated cylinders of 25 mL, all samples were shaken for 1 min, and placed on a table to rest during 1 min, and a photo was taken showing samples side by side, for comparison purpose. Optical microstructure was observed using an optical microscope (Carl Zeiss Jenaval, Carl Zeiss Microimaging GmbH, Germany) with an 20x, 40x or 100x objective and 10x optovar, coupled to a digital camera and software (EDN2 Microscopy Image Processing System). Before the observation, a small amount of sample was carefully placed on a glass slide, and placed a droplet of distilled water and covered with a cover glass.

2.4. Particle Size Distribution (PSD) analysis

PSD was evaluated by light scattering (Malvern Mastersizer 2000 with Hydro 2000s, Malvern instruments Ltd, UK). A little amount of dry sample was slowly added into a sample compartment, previously filled with ethanol (99.9%; room temperature), until obscuration reaches values around 10. The mean diameter was evaluated based for both particle volume (D4.3; Equation (1)) and the particle surface area (D3.2; Equation (2)). This is useful since the particles are not ideal spheres, and D3.2 is more influenced by smaller particles, whilst D4.3 is more influenced by larger ones (Bengtsson & Törnberg, 2011; Lopez-Sanchez et al., 2011). These analyses were carried out in triplicate.

\[
D_{4.3} = \frac{\sum n_i d_i^4}{\sum n_i d_i^3} \\
D_{3.2} = \frac{\sum n_i d_i^3}{\sum n_i d_i^2}
\]

2.5. Differential Scanning Calorimetry (DSC)

Thermal transition of starch samples was evaluated using a DSC (TA Instruments, New Castle, DE, USA). Samples (control and processed) were weighed (3 μg) in aluminium pans and water (7 μL) was added. Then, the pans were sealed, rested for 30 min at room temperature and heated at temperatures from 30 °C to 95 °C at the rate of 10 °C min⁻¹. An empty pan was used as reference, and the DSC equipment was calibrated with indium for temperature and heat capacity. From the DSC data it was obtained the thermal transitions of starch dispersions - according to the parameters Tg (onset), Tp (peak gelatinization) and Tc (conclusion) - AH value (referred to enthalpy of gelatinization), temperature range (ΔT = Tc - Tg) and degree of gelatinization (%G), that was calculated using the following Equation (3) (Blaszczak et al., 2007):

\[
%G = \left( \frac{\Delta H_{cs} - \Delta H_{ps}}{\Delta H_{cs}} \right) \cdot 100\%
\]

Where ΔHcs and ΔHps were the gelatinization enthalpies of control starch and pressurized starches, respectively.

2.6. Pasting properties

The pasting properties of pea starch samples were evaluated according to the method 162 of ICC (1996) using a Rapid Visco Analyser (RVA) (model RVA 4500, Peter Instruments, Warrewood, Australia) and the curves were analysed by the software TCW315.1255 through the profile ‘Extrusion-1’ and 3.0 g (db) of starch sample. The parameters analysed were the viscosity (cp) and the pasting temperature (°C). The ‘Extrusion-1’ profile was chosen by analysing the cold viscosity.

2.7. Statistical evaluation

When relevant, the effect of pressure was evaluated by using the
3. Results and discussion

3.1. Visual observation and optical microscopy

Fig. 1 shows a picture of pea starch processed HPP in water dispersion taken immediately after the process. Fig. 1 clearly illustrates that the high pressure process can affect the pea starch dispersion in water. It was observed that process up to 400 MPa did not change the appearance of the dispersion. However, after pressurization at 500 MPa, the sediment phase presented a higher volume, and, after process at 600 MPa, it was clearly observed a gel phase. This indicates that the HPP can lead to formation of pea starch gel at room temperature, and this process can be called ‘cold-gelatinization’ as did not require a conventional cooking step.

According to Santos et al. (2014), high pressure can increase the water hold capacity (WHC) of starch. The authors found that maize starch at 500 MPa for 15 min showed a 35-fold higher WHC, than the non-pressured sample. On the contrary, at 300 MPa sample did not show difference with the non-processed, being the results compatible with the obtained in the present research.

The results of starch pressurized dispersed in ethanol (data not showed) highlighted the importance of the water to promote gelatinization process. Fig. 2 shows the optical microscopy using regular and polarized light of pea starch dispersed in water. It can be noticed that no visual changes were observed for the ethanol dispersion pressurized up to 600 MPa.

Fig. 3 shows the optical microscopy only for the control and 600 MPa processed samples on ethanol dispersion, since the other did not show any difference.

As can be seen in Fig. 2, as pressures increases more effects can be noticed in the starch granules. Control sample showed the granules nearly rounded shaped on regular light, while on polarized light was observed a “Maltese-cross”, due to the starch that was not gelatinized. For samples processed up to 400 MPa, no differences between the shape of the granules, size and also the presence of “Maltese-cross” where observed in comparison with control sample. At 500 MPa, granules showed an increase in size, and became a swollen granule, at polarized light almost none region of the granule showed a difference in birefringence. Finally, at 600 MPa, granules were reduced and converted from a rounded shape to an irregular shape, and at polarized light, the starch showed no birefringence pattern. These results are in accordance with Fig. 1, corroborating the hypothesis that the HPP promotes slight and intense ‘cold-gelatinization’ at 500 and 600 MPa, respectively.

For sample dispersed in ethanol (Fig. 3) starch structure was preserved even after process at 600 MPa, with granule rounded shaped and similar size of the non-processed samples. The differences between the results obtained for starch dispersed in water and ethanol clearly indicates that the ‘cold-gelatinization’ caused by HPP occurred by forcing water molecules into the granule, promoting a hydration process of starch without heating, which at 600 MPa leads to complete gelatinization.

Several authors evaluated microscopically the impact of HPP on other sources of starch (Bauer et al., 2004; Blaszczyk, Fornal, Valverde, & Garrido, 2005; Li et al., 2015; Oh, Pinder, Hemar, & Anema, 2008; Pei-Ling, Qing, Qun, Xiao-Song, & Ji-Hong, 2012; Santos, Saraiva, & Gomes, 2015; Vallons & Arendt, 2009), Santos et al. (2015) did not verify major change in maize starch processed at 400 MPa at 27 °C for 5 min. Generally, gelatinization occurs between 450 and 600 MPa for different types of starch (red adzuki bean – Li et al., 2015, sorghum – Vallons & Arendt, 2009, tapioca – Bauer et al., 2004; Oh et al., 2008; Le Bail et al. 2013, waxy corn starch – Pei-Ling et al. 2012; Oh et al., 2008, rice, waxy rice and corn – Oh et al., 2008, pea starch – Le Bail et al. 2013, and broad bean – Le Bail et al. 2013). However, starch from potato did not change its birefringence after 500 MPa (Le Bail et al. 2013) or even after 600 MPa (Oh et al., 2008).

The differences of starch resistance to gelatinization at high pressure can be attributed to the content of amylose in the granule. Comparing the results of gelatinization of rice, waxy rice, corn, waxy corn, tapioca and potato, Oh et al. (2008) observed that gelatinization was higher for samples with low amylose content (waxy rice, waxy corn and tapioca). Similarly, Blaszczyk, Fornal, et al. (2005) verified that a sample with 68% of amylose did not show changes on birefringence and on the shape or size of granules after processing at 650 MPa for 9 min, but observed complete loss of birefringence after process at 650 MPa for 3 min for sample with traces of amylose. Therefore, as for thermal gelatinization, starch with low amylose content requires low energy input (pressure) to gelatinize.

The pea starch studied has intermediate amylose content (33%), therefore it has some level of ‘cold-gelatinization resistance’. This explains the small regions of birefringence observed for sample processed at 500 MPa. Le Bail et al. (2013) studied HPP gelatinization induced of several starches, included pea starch, and observed a similar result as in this work, with extend, but not total, level of birefringence. In the referenced studied, the observed effect was not dependent on the process temperature (20 or 40 °C).

The results observed for samples processed at 500 and 600 MPa are in accordance with the two-step mechanism proposed by Rubens, Snauwaert, Heremans, and Stute (1999), which described that the high pressure firstly hydrate the amorphous region of the granules, and this induces a swelling of granules, that promotes a distortion and further destruction of the crystalline region of the granule. On the second step, the crystalline region is easily accessed by water, and more water penetrate into the granule, increasing the swelling and hydrogen bond formation between water and starch, finalizing the gelatinization process.

3.2. Particle Size Distribution (PSD)

The effect of HPP on mean particle diameter of pea starch on water and ethanol dispersion are presented on Fig. 4. It can be noticed that, in general, the particles in water dispersion presented a higher mean diameter than particles in ethanol dispersion, corroborating the requirement of water dispersion to promote starch gelatinization under pressure.
Fig. 2. Optical Microscopy of Pea starch processed by HPP in water dispersion—comparison between pressures at 0 MPa — a, 300 MPa — b, 400 MPa — c, 500 MPa — d and 600 MPa — e.
Regarding the starch in water dispersion, the mean particle size showed no difference up to 400 MPa, an increasing at 500 MPa and then a slightly reduction at 600 MPa, but remaining higher than the size after processes up to 400 MPa. Regarding starch in ethanol dispersion, as stated above, no effect was observed in mean particle diameter with the increase of pressure level ($P < 0.05$).

The PSD of pea starch in both water and ethanol dispersion, after several high pressure conditions (conditions that particle size is significantly different from each other) is presented in Fig. 5. Again, is possible to notice that samples in ethanol dispersion showed no impact due to pressure and that only processes carried out in water dispersion at pressures above 500 MPa showed differences with the control sample. Starch size in ethanol dispersion showed a monomodal distribution, which confirms the fact the both $D[4,3]$ and $D[3,2]$ were equivalent. The distribution of particles in water dispersion showed a monomodal distribution with an agglomeration of larger particles, especially at 500 and 600 MPa. This explains $D[4,3] > D[3,2]$ for all samples in water dispersion, since larger particles are associated with higher values of $D[4,3]$. For non-processed samples and processed samples up to 400 MPa, these larger particles can be attributed to starch particles hydration that was induced in the 24 h of stirring preparation, since at room temperature the starch can increase its weight by approximately 30% of water (Biliaderis, Page, Maurice, & Juliano, 1986). For samples processed at 500 and 600 MPa, the increase in particle size can be attributed to the effect of pressure making more water to enter the granule, leading to gelatinization.

Pei-Ling et al. (2012), verified that high pressure processing did not affect significantly the mean particle size of tapioca and waxy corn starches, however, the distribution became broader. Using SEM (Scanning Electron Microscopy) Li et al. (2015) estimate the size of red adzuki bean starch granules processed at high pressure up to 450 MPa and observed that higher pressure samples showed partially disintegrated clusters and bigger size, which is similarly to pea starch results.

As observed, the presence of water clearly has a fundamental role on this phenomenon. This statement is reinforced by the already described two steps mechanism proposed by Rubens et al. (1999) and by the results of the study performed by Kawai, Fukami, and Yamamoto (2012), who verified the impact of several ratios (%) of starch/water on the gelatinization induced by HPP. At concentrations up to 30%, the authors observed that the gelatinization enthalpy decreased with the pressure and temperature increase, which are similar to diluted starch systems used in this work. At ratios of 40% and 50%, the temperature did not show any impact, however, the pressure still induced a reduction on gelatinization enthalpy. At ratio higher than 60% no gelatinization and, consequently, no impact on gelatinization enthalpy was observed even at 70°C and 1000 MPa. There was no sufficient water on those samples to hydrated the amylopectin, thus interfering in the melting process and consequently, unable the gelatinization. Pea starch at ethanol dispersion faced the same effect, without water the amylopectin did not hydrolyzed and would not gelatinize in this dispersion.

### 3.3. Pasting properties

The pasting properties of pea starch in water dispersion processed by HPP are presented in Fig. 6. The samples at ethanol dispersion...
dispersion were not presented since no effect was observed after HPP processing. Table 1 shows several parameters obtained by RVA equipment. It is notable the effect of high pressure in pea starch in water dispersion, in the same pattern: almost no effect up to 400 MPa; different behaviours after process at 500 MPa and 600 MPa.

The ‘Cold peak’, that represents the suspension viscosity observed by the equipment before heating, is approximately 65% higher for sample processed at 500 MPa comparing to the cold viscosity of the samples processed up to 400 MPa and 600 MPa. The ‘Cold peak’, that represents the suspension viscosity observed by the equipment before heating, is approximately 65% higher for sample processed at 500 MPa comparing to the cold viscosity of the samples processed up to 400 MPa and 600 MPa. This was expected since the PSD and mean diameter are higher for sample processed at 500 MPa, leading to an increase in the friction between particles with consequent increase in the viscosity. Also the sample processed at 600 MPa showed gelatinization prior RVA analysis, which explains the consistency 4 times higher for pea starch processed at 600 MPa when compared to the control sample.

Oh et al. (2008), observed a similar trend for starch of rice, corn, waxy corn and tapioca, with no changes on the initial viscosity for samples processed at 400 MPa and increase of viscosity after process at 600 MPa. Using sorghum starch dispersion, Vallons and Arendt (2009) also found that the initial complex viscosity was higher for samples processed at pressures higher than 500 MPa.

The ‘Raw peak’ also known as ‘peak viscosity’, is the maximum viscosity of the solution that occurs when the starch granules are completely hydrated and reach their maximum size, immediately before the granules start to break in minor particles (Kaur, Fazilah, & Karim, 2011). This is considered a good parameter to indicate the thickening properties of the starch. The samples up to 400 MPa showed a lower viscosity at the maximum point than samples processed at 500 and 600 MPa (which did not show statically difference among them; p < 0.05).

The ‘Final viscosity’ is the consistency obtained when the system is cooled after the end of the gelatinization process. Again, the sample processed at 600 MPa showed the highest value (~20% higher) and all other samples showed similar lower values (Table 1). Therefore, it can be concluded the HPP, especially at 600 MPa, changes the pasting profile of the pea starch improving its characteristics and possibly opening alternatives for industrial application of this starch.

Other authors who evaluate the pasting profile of high pressure processed starch observed that pressurization reduces the viscosity (peak and/or final viscosity) of red adzuki bean starch (Li et al., 2015), waxy corn and tapioca starch (Pei-Ling et al., 2012). The source of the starch, the amylose content and the drying method of
the analysis of the curves of Fig. 7.

Table 2 indicates some parameters obtained by DSC. Table 2

Table 1

<table>
<thead>
<tr>
<th>Pressure</th>
<th>Cold peak</th>
<th>Raw peak</th>
<th>Hold</th>
<th>Breakdown</th>
<th>Final viscosity</th>
<th>Setback</th>
<th>Peak time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>8.00 ± 0.01a</td>
<td>302.67 ± 3.40ab</td>
<td>93.67 ± 2.62c</td>
<td>209.00 ± 5.72a</td>
<td>284.33 ± 3.09b</td>
<td>190.67 ± 0.94b</td>
<td>6.16 ± 0.08a</td>
</tr>
<tr>
<td>300 MPa</td>
<td>8.33 ± 0.47a</td>
<td>285.67 ± 5.44a</td>
<td>89.00 ± 2.16bc</td>
<td>196.67 ± 7.41a</td>
<td>267.00 ± 2.16a</td>
<td>178.00 ± 0.01a</td>
<td>6.09 ± 0.06a</td>
</tr>
<tr>
<td>400 MPa</td>
<td>9.00 ± 0.82a</td>
<td>315.33 ± 2.87b</td>
<td>94.67 ± 0.47c</td>
<td>220.67 ± 3.30a</td>
<td>288.00 ± 0.82b</td>
<td>193.33 ± 1.25b</td>
<td>6.18 ± 0.03a</td>
</tr>
<tr>
<td>500 MPa</td>
<td>14.00 ± 0.82b</td>
<td>471.00 ± 15.94c</td>
<td>80.33 ± 1.89d</td>
<td>390.67 ± 17.56b</td>
<td>272.67 ± 3.86ab</td>
<td>192.33 ± 5.31b</td>
<td>6.13 ± 0.05a</td>
</tr>
<tr>
<td>600 MPa</td>
<td>34.00 ± 0.01c</td>
<td>455.33 ± 2.05c</td>
<td>82.33 ± 3.30ab</td>
<td>373.00 ± 5.35b</td>
<td>331.00 ± 6.16c</td>
<td>250.67 ± 2.87c</td>
<td>6.22 ± 0.03a</td>
</tr>
</tbody>
</table>

Fig. 7. Differential Scanning Calorimetry of Pea Starch Processed by HPP in water dispersion.

each study can explains the difference between these studies and the results obtained for the pea starch.

3.4. Differential Scanning Calorimetry (DSC)

Fig. 7 presents the DSC curves of pea starch processed by HPP in water dispersion. Table 2 indicates some parameters obtained by the analysis of the curves of Fig. 7.

The curves obtained for samples processed up to 400 showed a peak of ΔH, but samples processed at 500 MPa and 600 MPa did not show any peak, indicating that higher pressures (≥500 MPa) induce total gelatinization of the starch. However, no difference was observed between the control and the processed sample at 300 MPa, indicating that process at 300 MPa is unable to promote any degree of gelatinization. Same trend was observed on sorghum starch processed at 300 MPa for 10 min (Vallons & Arendt, 2009). The sample processed at 400 MPa showed a slightly reduction of the enthalpy, indicating 31% of gelatinization. For this sample HPP did not change the pea starch gelatinization temperature, since the temperatures were similar for non-processed and processed sample at 400 MPa.

Incomplete gelatinization was observed on waxy maize (81%) at 400 MPa for 30 min (Ahmed, Singh, Ramaswamy, Pandey, & Raghavan, 2014), potato starch (65% and 73%) processed at 600 MPa for 2 and 3 min, respectively (Blaszczack, Valverde & Fornal, 2005). Sorghum starch showed 34% and 83% at 400 MPa and 500 MPa, respectively, for 10 min (Vallons & Arendt, 2009), waxy corn starch dispersion (30% and 50%), processed at 450 MPa for 30 min (Pei-Ling et al., 2012) showed respectively 84.5% and 95.5% of gelatinization, and red adzuki bean (6%, 9% and 56%) processed at 150 MPa, 300 MPa and 450 MPa (Li et al., 2015). For these same studies, but using higher pressures and holding times, it was reached total starch gelatinization in the different sources of starch: tapioca starch and waxy maize at pressure conditions higher than 400 MPa and 600 MPa for 30 min, respectively (Ahmed et al., 2014), red adzuki bean at 600 MPa (Li et al., 2015), Tapioca at 600 MPa for 30 min (Pei-Ling et al., 2012) and sorghum starch at 600 MPa for 10 min (Vallons & Arendt, 2009). The different starch sources, starch types, amylose and amylopectin content and the process condition (time, temperature and pressure) explain these differences observed in the degree gelatinization induced by HPP.

Additionally to changes on the degree of gelatinization, some studies described that HPP was able to modify the temperature of gelatinization, such as on red adzuki bean starch (Li et al., 2015), potato starch (Blasszcak, Valverde et al., 2005 & Le Bail et al., 2013), modified tapioca and modified waxy maize starch (Ahmed et al., 2014), tapioca (Pei-Ling et al., 2012 & Le Bail et al., 2013), waxy corn (Pei-Ling et al., 2012), broad bean (Le Bail et al., 2013) and maize (Santos et al., 2015). This change can be associated with the type of starch and was not observed in this study. However, Le Bail et al. (2013) observed this phenomenon for pea starch, with increase of 5 and 8 °C on the gelatinization temperature after processing samples at 500 MPa for 20 and 500 MPa however at 40 °C, respectively. The observed differences among these results and the obtained in the present research can be explained once in our study the sample were freeze dried before DSC analyses, and on the referenced study the dispersion was analysed after the pressurization.

High pressure can destroy, or at least damage, the ordered structure of the starch granule, as can be noticed by the change in the enthalpy, which is related to phase transition (Liu et al., 2009). Although the complete vitreous transition induced by pressure was observed for samples pressurized at 500 MPa and 600 MPa (ΔH300 = 0), the results of the other assays (pasteing profile, microscopy and PSD) for these samples were different. This can be explained by the two step mechanism proposed by Rubens et al. (1999), which described that the water needs to enter in the

Table 2

<table>
<thead>
<tr>
<th>Sample</th>
<th>$T_{f_{\text{onset}}}$ (°C)</th>
<th>$T_{\text{peak}}$ (°C)</th>
<th>$T_{\text{end}}$ (°C)</th>
<th>$\Delta H$ (J/g)</th>
<th>$\Delta T$ (°C)</th>
<th>%G</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>53.61 ± 0.44a</td>
<td>58.79 ± 0.73a</td>
<td>62.78 ± 0.50a</td>
<td>3.75 ± 0.06a</td>
<td>9.17 ± 0.17a</td>
<td>0</td>
</tr>
<tr>
<td>300 MPa</td>
<td>53.33 ± 0.06a</td>
<td>56.33 ± 0.28a</td>
<td>62.22 ± 0.34a</td>
<td>3.79 ± 0.05a</td>
<td>8.88 ± 0.40a</td>
<td>0</td>
</tr>
<tr>
<td>400 MPa</td>
<td>53.73 ± 0.14a</td>
<td>58.34 ± 0.45a</td>
<td>62.08 ± 0.27a</td>
<td>2.57 ± 0.05b</td>
<td>8.36 ± 0.36a</td>
<td>31</td>
</tr>
<tr>
<td>500 MPa</td>
<td>Not detected</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>600 MPa</td>
<td>Not detected</td>
<td></td>
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<td>100</td>
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</tbody>
</table>

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granule, creating more hydrogen bonds, increasing the swelling and effectively inducing gelatinization in the second step. Therefore, the difference between samples processed at 500 MPa and 600 MPa is that, although 500 MPa is enough to change the organization of the pea starch and, consequently, its enthalpy, it is not a pressure high enough to make the complex “starch + water” to interact in order to achieve the complete gelatinization, with the maximum volume reduction foreseen by the Le Chatelier’s principle (Doustals, Marechal, Coquille, & Gervais, 1996). This lower volume probably was just reached after process at 600 MPa for 15 min for pea starch, characterized by the results obtained for the optical microscopy and pasting profile.

The results of all analysis performed indicated that all methodology employed are adequate to evaluate any direct or indirect effect on the pea starch caused by HPP. Even the more simple methodology (visualization in graduated cylinders) gives a good idea about the impact of the process on the starch. The microscopy and PSD allows a structural evaluation of the granule, improving the discussion of the changes that occurs at each pressure level. The DSC is the most sensitive method to evaluate the degree of gelatinization, quantifying effects even for the samples processed at 400 MPa (for which no other methodology showed alteration). On the other hand, this methodology cannot be used isolated to evaluate the impact of HPP in starches once the results obtained by DSC did not show difference between 500 and 600 MPa processed samples while in the other methodologies such as pasting profile, these two process conditions resulted in modified starch with different properties.

4. Conclusions

The high pressure processing caused the loss of the birefringence of the pea starch granules in water dispersion, changed the starch granule shape, size and particle size distribution, and the pasting properties. The relevant changes occurred with samples pressurized at 500 MPa/15 min, and the more significant changes occurred after process at 600 MPa/15 min. All changes observed reflect the starch gelatinization induced by the high pressure processing. By these results, it was concluded that HPP can be used as a physical methodology to modify pea starch (improving its performance and industrial application) and that the resistance of this kind of starch to HPP is similar to the starch from rice, corn, waxy corn and tapioca.

Additionally, these results are useful to understand the phenomenon of gelatinization induced by HPP, which forces the entrance of water inside the starch granule, promoting the “cold gelatinization” just for starch dispersed in water. No physical change on the granule occurs in absence of water, even at 600 MPa, making the process completely ineffective at this condition.

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